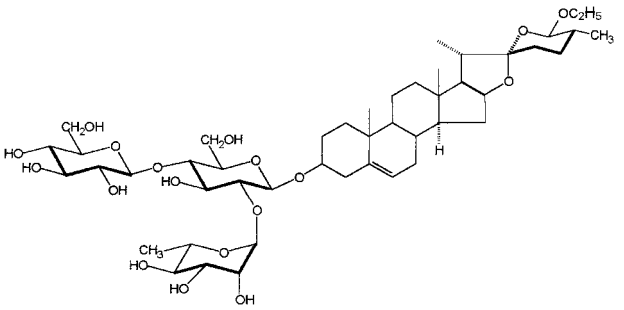

compound **1**

glucose and one molecule of rhamnose in their saccharide chain. This paper deals with the isolation and structural elucidation of a new steroidal saponin (**1**) from the ethanolic extract of the bulbs of *Lilium candidum* L. The new compound was separated chromatographically and characterized by ^{1}H, ^{13}C NMR, and MS and identified as (25*R*, 26*R*)-3β-{β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyloxy}-26-ethoxy-spirost-5-ene.

Experimental

The m.p. was measured on a Kofler micro hot-stage.

1. Equipment

MS were recorded on ZAB-EQ instrument (Micromass, Manchester, U.K.) using fast atom bombardment (FAB) with a glycerol matrix and Xe at 8 kV as a bombarding gas. Daughter ion linked scans at B/E = const. and parent ion linked scans at B^2/E = const., were used to determine the sequence of saccharides and the molecular weight of the aglycon. NMR spectra were recorded on a FT-NMR spectrometer Varian UNITY-500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz) in CD_3OD. For CC silica gel (Silpearl Kavalier Votice) was used. TLC was carried out on UV 254 or 366 plates and silica gel 60 F_{254} glass plates (Merck).

2. Plant material

Bulbs of *Lilium candidum* L. were collected near Bratislava, Slovak Republic.

3. Extraction and isolation

Fresh bulbs of *Lilium candidum* L. (1.7 kg) were extracted with EtOH at room temperature. The ethanolic extract was concentrated *in vacuo* (89 g) and partitioned between n-BuOH and H_2O (1 : 1). The butanolic layer was concentrated *in vacuo* and chromatographed over silica gel (Silpearl Kavalier Votice) with a mixture of $CHCl_3$ and MeOH (9 : 1), with increasing MeOH contents. A total of 82 fractions (100 ml) were collected.
Fractions 35–37 were combined and evaporated *in vacuo* and the residue was chromatographed over silica gel with the same solvent system as for the previous fraction to give compound **1** (30 mg), m.p.: 206–208 C. Standard FAB MS: m/z (% rel.int.): 951 (81) $[M + Na]^+$, 929 (5) $[M + H]^+$, 883 (22) $[M + H{-}C_2H_5OH]^+$, 825 (9), 737 (5) $[M + H{-}C_2H_5OH{-}Rha]^+$, 721 (4) $[M + H{-}C_2H_5OH{-}Glc]^+$, 441 (10) $[Aglycon + H{-}H_2O]^+$, 413 (35) $[M + H{-}C_2H_5OH{-}Rha{-}Glc{-}Glc]^+$, 395 (72) $[M + H{-}H_2O{-}C_2H_5OH{-}Rha{-}Glc{-}Glc]^+$, 253 (100). For ^{1}H and ^{13}C NMR data see Table.

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References

1 Haladová, M.; Eisenreichová, E.; Mučaji, P.; Buděšínský, M.; Ubik, K.: Collect. Czech. Chem. Commun. **63**, 205 (1998)
2 Haladová, M.; Eisenreichová, E.; Mučaji, P.; Buděšínský, M.; Ubik, K.: Pharmazie **54**, 159 (1999)

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Sesquiterpenoids from *Scorzonera hispanica* L.

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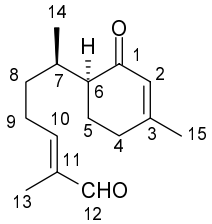
Scorzonera hispanica L. is a perennial herb, which is native to Southern Russia, the Ukraine, Kazakhstan, Eastern Central, South Eastern and South Western Europe [1]. In Central Europe it is widely cultivated as a vegetable and in former times it was also used in folk-medicine as a mucolytic [2]. In our continuing study of the phytochemistry of the Lactuceae tribe of the Asteraceae family we reinvestigated the constituents of *S. hispanica*. Prior studies led to the isolation and identification of 3,4-dimethoxy-cinnamic acid methyl ester, β-sitosterol, the lignan (3a*R*)-1*c*,4*c*-bis-4β-D-glucopyranosyloxy-3,5-dimethoxy-phenyl-(3ar,6ac)-tetrahydro-furo-3,4-*c*-furan as well as the sesquiterpenoid scorzoneroside [3–5].
Repeated CC and subsequent semi-preparative HPLC of methanol extracts of subaerial parts of *S. hispanica* yielded compounds **1**–**3**. The bisabolane derivative puliglutone (**1**) was identified on the basis of its ^{1}H NMR, ^{13}C NMR and HMBC spectra and in comparison with ^{1}H NMR data given in the literature [6]. This substance has been reported from the Asteraceae genera *Senecio*, *Oldenburgia* and *Pulicaria*, but up to now neither from the genus *Scorzonera* nor from any other genus of the Lactuceae [6–8]. As ^{13}C NMR data for compound **1** have not been published yet, they are given in the experimental section. Compound **2**, could be identified by ^{1}H NMR, ^{13}C NMR and HMBC experiments as ixerisoside D, which represents the 11,13-dehydro-derivative of scorzoneroside [9]. This substance has been isolated from *Ixeris repens*, an Asian species of the Lactuceae tribe, subtribe Crepidinae [9].
The ESIMS of compound **3** showed quasimolecular ion peaks at m/z 526 $[M + NH_4]^+$ and 509 $[M + H]^+$. HRFABMS established the molecular formula of $C_{26}H_{36}O_{10}$ showing a signal at m/z 509.2381 $[M + H]^+$

1



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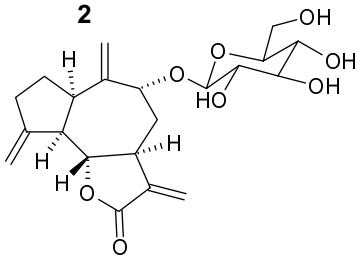

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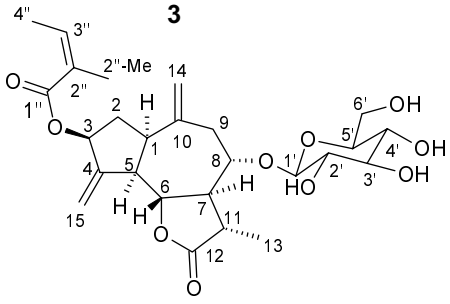

(calculated for $C_{26}H_{37}O_{10}$, 509.2387). The ^{1}H NMR and ^{13}C NMR data revealed the presence of a sesquiterpene lactone glycoside esterified with angelic acid. ^{1}H NMR signals of two exocyclic methylene groups (δ_H 5.36, br s, 5.24 br s and 5.08 br s, 5.01 br s), three oxygene bearing methine groups [δ_H 5.63 (dd, $J_{3,2b} = 8.5$ Hz, $J_{3,2a} = 6.5$ Hz), δ_H 4.12 (dd, $J_{6,5} = J_{6,7} = 9.5$ Hz) and δ_H 3.79 (m)], three tertiary protons (δ_H 3.04, 2.90 and 2.74), two pairs of methylene protons (δ_H 2.47, δ_H 1.85 and δ_H 2.93, δ_H 2.43) as well as a methyl group (δ_H 1.41) were assignable to the aglycon moiety (Table 1). The glycoside nature of the compound was supported by a doublet at δ_H 4.45 ($J_{1',2'} = 7.5$ Hz) assignable to the anomeric proton of β-glucose. The angelic acid moiety was indicated by signals of a vinylic proton [δ_H 6.15 (qq, $J_{3'',4''} = 7.5$ Hz, $J_{3'',2''\text{-Me}} = 1.5$ Hz) and two methyl groups (δ_H 1.98, δ_H 1.90). ^{13}C NMR experiments revealed 26 signals, three assignable to methyl groups, five to methylene groups, 13 to methine groups and five to quarternary carbons. The sesquiterpenoid moiety was identified by comparison of its ^{1}H and ^{13}C NMR data (Table) with those reported in the literature for 11β,13-dihydrodesacylcynaropicrin [10–12]. Signal assignments were verified by HSQC and HMBC experiments and the relative configurations were established by a NOE experiment, which showed crosspeaks between protons H-1 and H-3, H-3 and H-5, H-6 and H-8 as well as H-8 and H-11. The location of the sugar residue in position O-8 of the aglycon was deduced from the HMBC experiment which showed crosspeaks between H-8 and C-1′ as well as between H-1′ and C-8. There were no crosspeaks between the sesquiterpenoid moiety and the butenoate moiety. However, acylation of O-3 was established by the appearance of the deshielded H-3 signal [$\delta_H = 5.63$, 1 H dd ($J_{3,2b} = 8.5$, $J_{3,2a} = 6.5$)] which was downfield shifted by about 1 ppm in comparison to the respective signal of 11β,13-dihydrodesacylcynaropicrin [10]. The structure of the acyl moiety was identified as angelic acid by ^{1}H NMR and ^{13}C NMR data (Table 1) [13]. Thus, compound **3** is 3-*O*-angeloyl-11β,13-dihydrodesacylcynaropicrin-8β-D-glucoside, which represents a new natural compound. The closely related 11β,13-dihydrodesacylcynaropicrin-8β-D-glucoside was previously found in the Asteraceae species *Saussurea affinis* Spreng. and *Cynara cardunculus* L. [11–12].

Table: NMR data of compound 3

Position	^{1}H NMR	^{13}C NMR
1	3.04 1 H, dd (8.0, 8.0)	45.7
2	2.47 1 H, dt (14.0, 6.5)	37.5
	1.85 1 H, m	
3	5.63 1 H, dd (8.5, 6.5)	76.0
4		150.9
5	2.90 1 H, dd (8.5, 6.5)	52.3
6	4.12 1 H, dd (9.5, 9.5)	81.1
7	2.27 1 H, ddd (9.5, 9.5, 9.5)	55.4
8	3.79 1 H, m	85.3
9	2.93 1 H, dd (14.0, 5.5)	44.3
	2.43 1 H, dd (14.0, 7.5)	
10		145.6
11	2.74 1 H, m	42.4
12		181.7
13	1.41 3 H, d (7.0)	16.8
14	5.08 1 H, br s	116.7
	5.01 1 H, br s	
15	5.36 1 H, br s	113.4
	5.24 1 H, br s	
1′	4.45 1 H, d (7.5)	105.3
2′	3.21 1 H, dd (7.5, 7.5)	75.5
3′	3.30 1 H, m*	78.7
4′	3.30 1 H, m*	71.6
5′	3.30 1 H, m*	78.0
6′	3.88 1 H, dd (11.5, 1.5)	62.8
	3.67 1 H, dd (11.5, 5.0)	
1″		168.0
2″		129.0
3″	6.15 1 H, qq (7.5, 1.5)	139.1
4″	1.98 3 H, dq (7.5, 1.5)	15.9
2″-Me	1.90 3 H, dq (1.5, 1.5)	20.7

* Overlapping signals

Experimental

1. Apparatus

NMR spectra were recorded on a Varian 500 spectrometer at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in MeOH-d_4.

2. Plant material

Scorzonera hispanica L. (black salsify) of Belgian origin (De Maeyer, Reg. Nr. 32134) was purchased at the local market in Innsbruck. A specimen was deposited at the Institute of Pharmacy.

3. Extraction and isolation

The subaerial parts of *S. hispanica* (584 g) were freeze-dried, ground and exhaustively extracted with methanol. The methanolic extract was concentrated in vacuo to give 195 g of extract. The extract was partitioned between water and ethylacetate. The EtOAc phase was brought to dryness in vacuo to give 34.8 g of residue. The residue was repeatedly chromatographed on silica gel [Merck Kieselgel 60 (40–63 m)], applying gradients of CH_2Cl_2–MeOH and CH_2Cl_2–$(CH_3)_2CO$.

Fractions containing predominantly **1** or **2** were finally purified by isocratic semi-preparative HPLC using a Merck 250 × 10 mm LiChrospher RP-18 (10 µm material) column as stationary phase and CH_3CN/H_2O mixtures (25/75; 50/50) as mobile phases. The flow rate was set to 3.5 ml/min and the detection wavelength to 205 nm. For each run 100 µl aliquots of solutions containing 10 mg/ml of the respective substances were injected to yield 14.5 mg of pure compound **1** and 4.1 mg of pure compound **2**.

Fractions containing predominantly compound **3** were purified by isocratic MPLC at a flow rate of 1.5 ml/min, using a column (20 × 1 cm) packed with Merck LiChroprep RP-18 material and a mixture of CH_3CN/H_2O (25/75) as mobile phase to yield 8.2 mg of **3**.

*4. ^{13}C NMR data of puliglutone (**1**)*

203.6 (C-1), 197.1 (C-12), 165.5 (C-3), 156.7 (C-10), 140.6 (C-11), 127.2 (C-2), 50.9 (C-6), 34.1 (C-8), 31.7 (C-7), 31.6 (C-4), 27.9 (C-9), 24.2 (C-15), 23.7 (C-5), 16.0 (C-14), 9.1 (C-13).

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References

1 Meusel, H.; Jäger, E. J.: Vergleichende Chorologie der Zentraleuropäischen Flora, Vol. 3, p. 534, G. Fischer Verlag, Jena, 1992
2 Siegmund, F.: Kräuterkunde, p. 529, Karafiat, Brünn, 1874
3 Tolstikhina, V. V.; Bryanskii, O. V.; Syrchina, A. I.; Semenov, A. A.: Khim.Prir.Soedin. **24**, 763 (1988)
4 Bryanskii, O. V.; Tolstikhina, V. V.; Semenov, A. A.: Khim. Prir. Soedin. **28**, 591 (1992)
5 Bryanskii, O. V.; Tolstikhina, V. V.; Zinchenko, S. V.; Semenov, A. A.: Khim. Prir. Soedin. **28**, 640 (1992)
6 Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H.: Phytochemistry **20**, 2389 (1981)
7 Zdero, C.; Bohlmann, F.: Phytochemistry **28**, 3345 (1989)
8 Mossa, J. S.; Muhammad, I.; El-Feraly, F. S.; Hufford, C. D.; McPhail, D. R.; McPhail, A. T.: Phytochemistry **31**, 575 (1992)
9 Warashina, T.; Ishino, M., Miyase, T.; Ueno, A.: Phytochemistry **29**, 3217 (1990)
10 Singhal, A. K.; Chowdhury, P. K.; Sharma, R. P.; Baruah, J. N.; Herz, W.: Phytochemistry 21, 462 (1982)
11 Das, S.; Baruah, R. N.; Sharma, R. P.; Baruah, J. N.; Kulanthaivel, P.; Herz, W.: Phytochemistry **22**, 1989 (1983)
12 Shimizu, S.; Ishihara, N.; Umehara, K.; Miyase, T.; Ueno, A.: Chem. Pharm. Bull. **36**, 2466 (1988)
13 Eliel, E. L.; Wilen, S. H.: Stereochemistry of Organic Compounds, p. 570, Wiley, New York 1994

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